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Research Article

Evaluation of Some Spermatological Characteristics in Denizli Cocks

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Summary: The aim of this research was to prove the principal spermatological characteristics by in vitro datas in Denizli cocks in Turkey. In this experiment, 31 Denizli cocks were used. Semen was collected from cocks by abdominal massage method, two times a week. The spermatological characteristics of samples are as following: Ejaculate volume average 0.70±0.01 ml, spermatozoa motility 72.32±0.80%, spermatozoa concentration 2.38±0.03x10^9/ml, percentage of abnormal spermatozoa 7.33±0.18%, percentage of live-dead spermatozoa 21.65±0.81% and pH 7.68±0.01. Acrosome, head, middle piece and tail deformations of 558 ejaculates had been collected were recorded as average 0.62±0.04%, 1.34±0.05%, 2.47±0.05% and 2.89±0.08%, respectively.

Key Words: Abnormal spermatozoa, concentration, Denizli cocks, motility.

Denizli Horozlarında Bazı Spermatolojik Özelliklerin Değerlendirilmesi

Özet: Bu araştırmanın amacı, Türkiye'ye ait bir ırk olan Denizli horozlarında başlıca spermatolojik özelliklerin in vitro bulgularla ortaya konulmasıdır. Bu çalışmada Denizli ırkı 31 horoz kullanılmıştır. Horozlardan spermalar haftada 2 kez abdominal masaj yöntemiyle alınmıştır. Horozlardan alınan sperma örneklerinin spermatolojik özellikleri; ejakülat miktarı 0,70±0,01 ml, spermatozoa motilitesi % 72,32±0,80, spermatozoa yoğunluğu 2,38±0,03x10^9/ml, anormal spermatozoa oranı % 7,33±0,18, ölü-canlı spermatozoa oranı % 21,65±0,81 ve spermatozoa pH değeri 7,68±0,01 olarak bulunmuştur. Çalışmada elde edilen toplam 558 ejakülatın anormal spermatozoa tiplerinden akrozom, baş, orta kısım ve kuyruğa ait bozuklukların genel ortalama değerleri sırasıyla %0,39±0,03, % 1,06±0,03, % 2,32±0,05 ve % 2,53±0,04 olarak belirlenmiştir.

Anahtar Kelimeler: Anormal spermatozoa, Denizli horozu, motilite, yoğunluk.

Introduction

Denizli fowl is a local breed, which belongs to Denizli province and surroundings in Turkey. Denizli cocks are famous for their voices and long crowing. Its voice and characteristics of crowing are high-pitched, deep and bass. There are different kinds of Denizli cock such as, single comb or rose, and colored or black feathers. Cocks have 5 different colors. There are black rings around their eyes. Their auricles are red or there is a piece of whiteness on the red part. The colour of hen's body feathers is black. The egg shell colour is white (1, 2, 23).

Approximately 3 billion spermatozoa are produced daily by a sexually active cock. The testes are located in the centre of the body cavity and, therefore, spermatogenesis proceeds at the internal body temperature of 41^{0}C in birds, as opposed to the scrotal temperature of 24-26^{0}C in mammals (10). The testes are attached by ligaments to the dorsal surface of the peritoneal cavity, adjacent to the adrenal glands and ventral to the kidneys. The dorsal surface of the testis is only a few millimetres from the ventral surface of the spine (10). Both testes are functional in the cocks. As sexual maturity is attained the weight of the paired testes increases from 2-4 g to 25-35 g; the left testis is usually 0.5-3 g larger than the right one. Semen is ejaculated from the phallus mixing with lymph like liquid (10, 14). Qualified and high concentration semen of cock is pink-white and consistency is also high. Ejaculation volume, which depends on breed, age, individual, season, light and many other environmental factors, is averagely 0.7 ml, spermatozoa concentration 3.0x10^9/ml and pH of spermatozoa 7.5 (8,10,26). First studies which were done to get semen from poultry were carried out in the early 20th century. In 1912, after killing a cock, Ivanof made artificial insemination (AI) with semen collected by pressure from ductus deferens. In the following years, too many studies were conducted on this subject, Burrows and Quinn collected semen by massage method (10,11,17). Alkan et al. (3) and Keskin et al. (14) found ejaculate volume for Erbro cocks averagely 3.29±1.19 ml and 0.6±0.1 ml, respectively. Kono and Hiura (15) found the ejaculate volume of Single Comb White cocks 0.1-1.1 ml. Lake and Stewart (16) who collected semen from cocks by massage method and Rouvier et al. (18) found the average ejaculate volume for broiler cocks 0.35 ml, for light-weight

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egg layer 0.15 ml, for medium-weight egg layer 0.2 ml. In the researches made with Leghorn cocks, ejaculate volume is averagely 0.55±0.05 ml (20), 0.07 ml (25) and 0.3 ml (7); for New Hampshire cocks this volume was 0.68±0.04 ml and 0.13 ml (20) and for Fayoumi, Plymouth Rock and Rhode Island breeds this volume was 0.26, 0.62 and 0.48 ml, respectively (12). Keskin et al. (13) found this volume for Denizli cocks 0.6±0.1 ml.

Keskin et al. (13) determined the spermatozoa motility in Denizli cocks as 65.0±2.9%. Different researchers who made researches on spermatological characteristics of cock semen found the spermatozoa motility for Erbro cocks 79.4±11.5 and 87.7±0.9% (14), for Leghorn breed 83.2±0.6%, for New Hampshire breed 77.6±0.2% (20); but Pakdil (17), Alkan et al. (3), Carvallo et al. (5), Chalah et al. (6), Dube et al. (9) and Schula and Tomar (22) have found 82.2%, 85.83±6.19%, 50.8%, 83%, 80% and 86.5%.

Keskin et al. (13) found spermatozoa concentration for Denizli cocks 2.0±0.2x10^9/ml. Some researchers found spermatozoa concentration 1.206±0.06x10^9/ml (17), 2.2x10^9/ml (14) for Erbros, 1.878±0.2x10^9/ml for Leghorn and 3.347x10^9/ml for New Hampshire (20), 2.20x10^9/ml (25), 5.7x10^9/ml for Broiler type stock and 5.0x10^9/ml for light-weight egg layer and medium-weight egg layer (18) and 3.218x10^9/ml (22).

Banarjee and Katpatal (4), who examined the changes in the quality of cock ejaculates, determined the rate of abnormal spermatozoa rate for White Leghorn, Rhode Island Red, Leghorn x Rhode Island Red and Deshi cocks breeds were 23.3%, 23.2%, 24.2% and 25.9% respectively. Keskin et al. (13) found this rate as 11.3±1.6 % for Denizli cocks. The abnormal spermatozoa rate for Erbro cocks found 5.0±0.06% (14). Sevinç et al. (19) fixed the total abnormal spermatozoa rate as 5.44±0.73% for Leghorns and 6.76±0.95% for New Hampshire. However, other researchers determined this rate as 5.25±0.55% (17), 11.83±0.96% (3) and 5.4±0.7% (27).

The semen pH value for Denizli and Erbro cocks found 7.5±0.1 7.4±0.1 (13,14). Sevinç et al. (20, 21) found this value as 6.9 for White Leghorn and New Hampshire. The live-dead semen rate was 83.34±6.43% (3) and 9.6±0.5% (27).

The studies have been still carried on for improving genetic characteristics of Denizli cocks and chickens which are covered in the scheme of indigenous animal species and breeds in Lalahan Livestock Research Center Institute. There is only one research about spermatological characteristics of Denizli cocks in Turkey. Therefore, the aim of this research was to get some statistical data about the spermatological characteristics of Denizli cocks and to contribute the future experiments. This research is an "Informative Study" about Denizli cocks semen.

Materials and Methods

Thirty one Denizli cocks aged 44 weeks, from the Denizli conservation flock in Lalahan Livestock Research Center Institute, were used in this study. The cocks were kept in individual battery typed cages under a day length of 16 h lightness program and ad libitum nutrition was applied. Semen was collected by abdominal massage method two times a week in the morning during 9 weeks (27 March / 25 May 2001) (10). Ejaculate volume was determined as 'ml' by finding directly from semen collecting tube. Semen was diluted by Laiciphose extender (1:4) (I:M:V; France) and motility was estimated. by a hot plate phase-contrast microscope (Nikon-Labophot, Japan) at x200 magnification. Spermatozoon concentration was calculated by using a digital photometer (Accuell Poultry Photometer, I.M.V., France) and was recorded as x10^9/ml. A hancock solution was employed in morphologic observations. The preparation was examined by light microscope at x1000 magnification by counting 200 cells (total). A drop of fresh semen was mixed with a drop of eosin and it was examined at 400x magnification and, about 200 spermatozoa were counted for dead-live spermatozoa. The pH value of the fresh semen was measured by using a colour-scaled pH meter (5.5 – 9.0, Merck) (10, 13, 26).

Statistical analysis of the spermatological characteristics of fresh semen was performed by the SPSS program and ANOVA for repeated measures. When the F values were significant ($p<0.05$), Duncan's multiple range test was performed.

Results

This informative study aimed to revealing the truth about the semen characteristics of Denizli cocks. The obtained results of this study are similar to the other studies which were carried out in the other breeds. In this informative study, general average values of Denizli cocks' spermatological characteristics are shown in Table 1. During these 9 weeks period, the differences in the

Table 1: Some spermatological characteristics for Denizli cocks (n=62).

Week	Spermatological Characteristics $\overline{X} \pm S_{\overline{X}}$					
	Ejaculate Vol. (ml)	**Motility (%)**	**Concentration (x109/ml)**	**Abnormal Sp. (%)**	**Dead Sp. (%)**	**PH**
1	0.66±0.02	74.27±2.43	2.26±0.09^{ab}	6.16±0.43^{a}	19.47±2.43	7.54±0.04^{a}
2	0.66±0.02	74.44±2.19	2.20±0.08^{a}	6.35±0.40^{a}	19.15±2.19	7.55±0.05^{a}
3	0.71±0.03	71.05±2.64	2.35±0.09^{abc}	7.01±0.47^{ab}	22.44±2.72	7.69±0.04^{b}
4	0.69±0.03	71.21±2.39	2.36±0.08^{abc}	7.59±0.50^{bc}	22.39±2.46	7.67±0.04^{b}
5	0.71±0.03	72.26±2.29	2.48±0.10^{bc}	7.54±0.54^{bc}	21.53±2.32	7.69±0.04^{b}
6	0.67±0.02	73.47±2.42	2.58±0.09^{c}	7.79±.57^{bc}	21.11±2.45	7.76±0.03^{b}
7	0.70±0.03	70.24±2.61	2.35±0.10^{abc}	8.04±0.65^{c}	23.74±2.60	7.71±0.03^{b}
8	0.70±0.03	70.56±2.58	2.40±0.11^{abc}	7.77±0.61^{bc}	23.82±2.62	7.78±0.03^{b}
9	0.76±0.03	73.39±2.31	2.46±0.09^{bc}	7.61±0.67^{bc}	21.21±2.28	7.75±0.03^{b}
General Average (n=558)	0.70±0.01	72.32±0.80	2.38±0.03	7.33±0.18	21.65±0.81	7.68±0.01
F	1,75	1,10	2,56*	4,96**	1,15	4,23**

* : p<0.05 ** : p<0.001
a-c : Values within each column with different superscripts differ significantly.

Table 2 : Average rate (%) of acrosome, head, middle part and tail defects at Denizli cocks

Week	Morphological Defects $\overline{X} \pm S_{\overline{X}}$			
	Akrozom (%)	**Head (%)**	**Middle (%)**	**Tail (%)**
1	0.38±0.08	1.11±0.08	2.59±0.17	2.06±0.19^{a}
2	0.61±0.12	1.30±0.11	2.21±0.13	2.24±0.19^{ab}
3	0.61±0.13	1.29±0.11	2.53±0.17	2.58±0.21^{bc}
4	0.67±0.14	1.51±0.17	2.72±0.13	2.71±0.22^{cd}
5	0.75±0.13	1.41±0.19	2.35±0.11	3.01±0.22^{cde}
6	0.69±0.14	1.37±0.17	2.58±0.15	3.14±0.26^{de}
7	0.66±0.15	1.48±0.18	2.46±0.15	3.46±0.29^{e}
8	0.62±0.14	1.33±0.19	2.37±0.15	3.43±0.28^{e}
9	0.56±0.14	1.25±0.20	2.40±0.14	3.38±0.28^{e}
General Average (n=558)	0.62±0.04	1.34±0.05	2.47±0.05	2.89±0.08
F	1,27	1,33	1,37	11,03**

** : p<0.001
a-e : Values within each column with different superscripts differ significantly.

spermatozoa motility, spermatozoa concentration, abnormal spermatozoa and pH values among the collected semen were considered statistically important ($p<0.05$ and $p<0.001$). While no difference was found in the results of ejaculate volume and dead-live spermatozoa among the weeks ($p>0.05$), some individual differences were determined among cocks ($p<0.05$ and $p<0.001$).

The general average values of defect found in the acrosomes, heads, middle parts and tails of totally 31 cocks were 0.62±0.04%, 1.34±0.05%, 2.47±0.05% and 2.89±0.08%, respectively (Table 2). At the end of the evaluation, a statistical difference was found depending on cock's spermatozoa's tail among the weeks ($p<0.001$). While no difference was found in the defects occured in acrosomes, heads and middle parts among the weeks ($p>0.05$), but some individual differences were determined among cocks ($p<0.05$ and $p<0.001$).

Discussion

Cocks show their desire to mate late in the afternoons. Semen of cocks is collected and AIs are done during those hours in the afternoon (13). But, in this research, the procedure was different because ejaculates were collected from Denizli cocks in the mornings. However, some other researchers had indicated semen-collecting hours were not so effective on the spermatological characteristics. On the other hand, the frequency of collection of semen was effective on spermatozoa (8,16,18). Among 558 ejaculates, the maximum ejaculate volume was recorded 1.5 ml (this amount was taken from number 30), and the minimum ejaculate volume was recorded 0.2 ml (for cocks number 1,2,13,24,27 and 30). The general average ejaculate volume was 0.70±0.01 ml and this volume was accepted within the physiological limits. In this informative study, similar values to those of Keskin et al. (13) were found. Sometimes spermatological characteristics of cocks passed over the normal value and sometimes rested under those limits. The general average of spermatozoa motility of 558 ejaculates was 72.32±0.80%, and for the cocks numbered 22 and 27 this percentage was found 10 % (especially for 27^{th} cock, this quantity is really very low compared to other cocks). But, we found lower motility rates than the ones determined by Keskin et al. (13). Semen-collecting hours and different researchers who carried out this experiment might cause the difference. The general spermatozoa concentration that depends on various factors such as breed, age, light, season, individual, semen-collecting frequency was determined as $2.38\pm0.03x10^9$/ml, and the minimum value was found as $360x10^6$/ml (for 27^{th} cock) and, the maximum value was determined as $3.94x10^9$/ml (for 11^{th} cock). Some other researchers (8,10,18) informed that body-weight had an effect on spermatological characteristics, and they also observed the ejaculate volume increase for heavy breeds and the spermatozoa concentration decrease. Unlike their research results, in our study, we didn't find any similarities between those characteristics.

As it is known that the morphological defects affect the fertility more than the motility can do in the evaluation process of semen quality. One of the most important criteria is to designate the spermatozoa having these types of structure (8, 10). In this experiment, at the end of the morphological examinations, general average was 7.38±0.18%. The maximum value was 34% for the 27^{th} cock. Many researchers indicate that defects depend on cock semen acrosome from the point of its effect on fertility, are the most important ones (16, 18). In this experiment, the average defects in total 558 ejaculates were sequentially: 0.62±0.04% for acrosome, 1.34±0.05% for head, 2.47±0.05% for middle part and 2.89±0.08% for tail. Average of total abnormal spermatozoa percentage for 31 cocks was 7.33±0.18%. Dead-live spermatozoa rate was determined as 21.65±0.81%. This was considered normal in its limits when compared to the spermatozoa motility rates. General average semen pH was calculated as 7.68. That was within its normal limits. These findings that we got from the experiments were quite similar to the ones that the other researchers found. In the evaluation of the sperm characteristics, the variations in the condition of the study (e.g. season of the year, temperature, photoperiod, age, breed, frequency of the ejaculation and technician differences in sperm examinations) might cause those differences in the outcomes of this study.

In conclusion, the outcomes obtained in the informative study which was considered as a pre-study, will help us do some further researches on freezing of Denizli cocks' semen and artificial insemination studies with frozen semen. This research was to help other experiments about the principal spermatological characteristics of Denizli cocks by revealing their in vitro outcomes.

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